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Citation for published version:

Pang, LY & Argyle, DJ 2009, 'Using naturally occurring tumours in dogs and cats to study telomerase and cancer stem cell biology', *BBA - Proteins and Proteomics*, vol. 1792, no. 4, pp. 380-91.
<https://doi.org/10.1016/j.bbadis.2009.02.010>

Digital Object Identifier (DOI):

[10.1016/j.bbadis.2009.02.010](https://doi.org/10.1016/j.bbadis.2009.02.010)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

BBA - Proteins and Proteomics

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Review

Using naturally occurring tumours in dogs and cats to study telomerase and cancer stem cell biology

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ARTICLE INFO

Article history:

Received 11 December 2008

Received in revised form 15 February 2009

Accepted 17 February 2009

Available online 28 February 2009

Keywords:

Dog

Cat

Stem cell

Telomerase

ABSTRACT

The recently described cancer stem cell theory opens up many new challenges and opportunities to identify targets for therapeutic intervention. However, the majority of cancer related therapeutic studies rely upon rodent models of human cancer that rarely translate into clinical success in human patients. Naturally occurring cancers in dogs, cats and humans share biological features, including molecular targets, telomerase biology and tumour genetics. Studying cancer stem cell biology and telomere/telomerase dynamics in the cancer bearing pet population may offer the opportunity to develop a greater understanding of cancer biology in the natural setting and evaluate the development of novel therapies targeted at these systems.

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1. Introduction: stem cells and cancer

Adult stem cells exist in essentially all mammalian tissues, and contribute to tissue repair and organ maintenance by replacing differentiated cells as they are lost through attrition or damage. These cells are capable of self-renewal and can give rise to all cell lineages of the tissue they reside in [1]. The loss of stem cell self-renewal capacity underlies certain degenerative diseases and appears to contribute to mammalian ageing [2]. However in long-lived organisms, self-renewing, highly undifferentiated stem cells may provide an attractive substrate for malignant transformation [3]. The cancer stem cell theory suggests that cancer originates from adult stem cells. Mutations in adult stem cells are more likely to have tumorigenic consequences than if the same mutations occurred in cells destined to die or differentiate [4]. For example, unrepaired genetic alterations in stem cells will be inherited by self-renewing daughter cells and hence these mutations will accumulate with aging. The process of tumorigenesis will be further aided by functional mutations that provide a growth or survival advantage and therefore a positive selection for the mutant stem cell clone. Further expansion of this stem cell clone and differentiation of mutant daughter cells would lead to a heterogeneous population of cancer cells, constituting a “tumour organ” (Fig. 1) [5]. Potent tumour suppressor mechanisms, such as senescence and apoptosis, exist to sense damaged stem cell genomes with malignant potential and limit replicative expansion or terminate such clones, respectively. However, fully-fledged cancer will result from the accumulation multiple

cancer-promoting events that enable cell immortality and bypassing of tumour suppressor mechanisms [6].

Cellular immortalisation is a critical step in carcinogenesis, enabling the cell to evade cellular senescence and acquire an infinite life span [7]. In the absence of immortalisation a cell may obtain essential oncogenic hallmarks, including self-sufficient growth, insensitivity to antigrowth signals, and evasion of apoptosis, but could not proliferate indefinitely [8,9]. This highlights that senescence is an important tumour suppressor mechanism by which cells can suppress aberrant growth by arresting cell proliferation. At the molecular level, mechanisms that contribute to a cell escaping senescence and becoming immortal include genomic instability; telomere length stabilisation; epigenetic gene silencing by selective promoter methylation; oxidative DNA damage; inactivation of cell cycle regulatory genes such as p16^{INK4a}, p53, RB or p21^{WAF1}; overexpression of cellular oncogenes such as cMYC or BMI-1; and expression of viral oncogenes [7,10,11]. Telomere shortening represents a cell intrinsic mechanism associated with senescence. Telomeres cap the end of each chromosome, and in most mammalian somatic cells, telomeres get progressively shorter with each round of cell division until they reach a critically short length. Upon which chromosomal ends become exposed and activates DNA damage responses that mediate cell cycle arrest or apoptosis [12,13]. In most cases, maintenance of telomere length is required for immortalisation and arises early in tumour progression [14]. In the absence of immortalisation a cell is unable to undergo malignant transformation. Therefore further understanding of the immortalisation process and how this applies to cancer stem cells could provide novel early molecular targets for the treatment and prevention of cancer.

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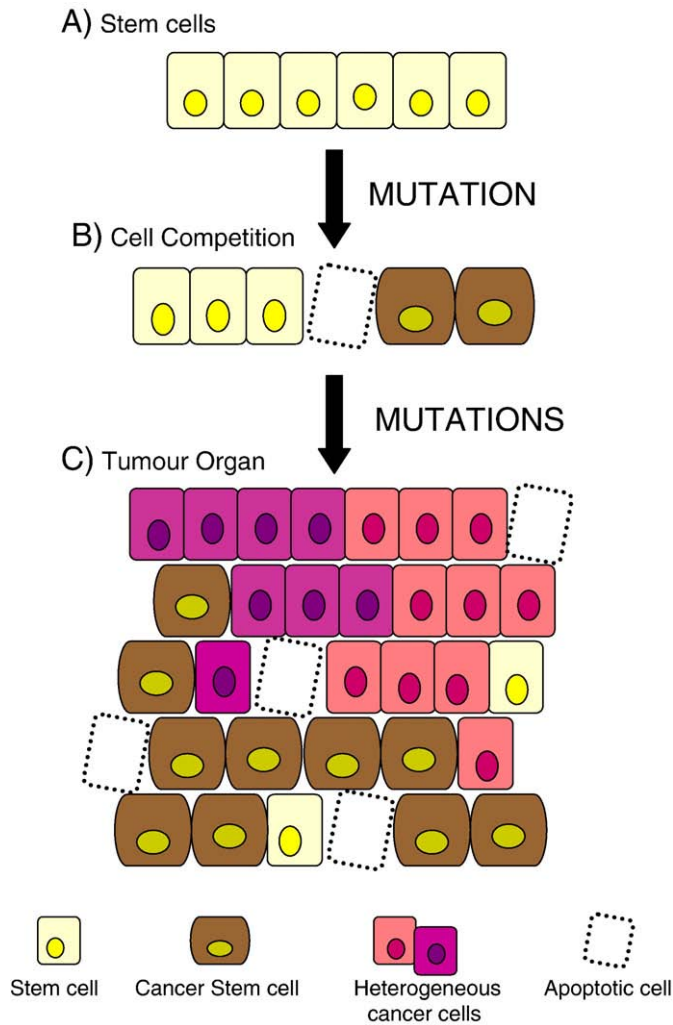


Fig. 1. Schematic representation of tumour development. (A) Stem cells acquire a mutation that provides a growth advantage. (B) Mutated stem cells can out grow non-mutated stem cells by cell competition. (C) Selection of additional mutations gives rise to the tumour organ consisting of a heterogeneous population of cancer cells.

One of the major hurdles to effective translation of our understanding of cancer biology into clinical practice has been the lack of good pre-clinical models of cancer. In this review, we will focus on the biology of cancer stem cells, telomeres and telomerase but suggest that by adopting a comparative approach to studying these systems in companion animal cancers, we may develop a pre-clinical model system that is more relevant to human oncology than current rodent models.

2. Comparative oncology

The development of rodent transgenic and xenograft tumour models has allowed major advances in our basic understanding of cancer biology in a very cost effective and timely fashion. However, a near universal reliance on these models has highlighted some major deficiencies. For example, implanted or induced tumours in xenograft models do not always behave in the same biological way as the natural parent tumour in humans, which is especially true of primary tumour growth and metastasis. In addition, treatments that demonstrate success in rodent models rarely translate into successful therapies in human clinical trials, clearly indicating fundamental differences that are difficult to resolve without utilizing alternative models [15]. As we move towards developing highly targeted therapies for specific cancer

types, these problems will become further exaggerated obviating the need to combine rodent studies with comparative cancer studies in other species to optimize the potential of developing the most effective cancer therapies for people [16].

The term comparative oncology is often used to describe the integration of studies in naturally occurring cancers in animals to the study of human cancer biology and therapy and the contribution of comparative studies to our understanding of human cancer cannot be overstated. Studies on avian, feline and bovine retroviruses underpinned the discovery of oncogenes and tumour suppressor genes and contributed to the discovery of HTLV-1 in man [17]. Seminal studies on the role of DNA papilloma viruses in carcinogenesis in cattle have directly facilitated the development of the first effective cancer vaccines for cervical cancer in women [18]. In recent years, and with the recognition of some of the disadvantages of defined rodent models, researchers have turned to naturally occurring tumours in dogs and cats to provide further insights into this devastating disease.

3. Dogs and cats as model systems

The improved life expectancy of people through control of infectious diseases and advances in public health has led to an apparent increase in age-related diseases such as cancer and osteoarthritis. A similar pattern has been observed in pet dogs and cats through vaccination regimes for once fatal infectious diseases and major advances in veterinary care. It is estimated that 1 in 3–4 dogs will develop cancer (and around 1 in 5 cats), and with around 7 million dogs in the UK this represents an opportunity to exploit these cancers in terms of identifying cancer-associated genes, studying environmental risk factors, understanding tumour progression and evaluating new therapies [19].

Naturally occurring cancers in dogs and cats share many features with their human counterparts (Table 1). Importantly, cancers in these species develop naturally and in the context of an intact immune system where tumour, microenvironment and host are syngeneic. For the most part tumour histologies observed in human medicine are represented in both dogs and cats and share biological behaviour [20]. Importantly, these observed similarities can be supported with hard genetic evidence with the publication of the canine genome and the increased portfolio of molecular tools (e.g. canine-specific affymetrix array) for this species [16,21]. It is becoming clear that the molecular drivers for both companion animal cancers and human cancers are analogous with near identical specific genetic changes in oncogenes and tumour suppressor genes. As an example, a recently constructed syntenic karyotype map between humans and dogs demonstrated strong similarities in cytogenetic abnormalities in Non-Hodgkin Lymphoma (NHL) occurring in both of these species [22].

Table 1

The similarities between human cancers and canine and feline cancers

Feature	Comment
Incidence	The incidence of cancer in dogs is reported to be 1 in 3–4 and 1 in 4–5 in cats, representing a potentially large study population.
Histology	The majority of human cancers are well represented in the canine population including breast cancer, melanoma, head and neck squamous carcinoma and osteosarcoma. Dogs and men are the only mammalian species where prostate carcinoma is recognised [16].
Biological behaviour	As with human cancer, cancer in dogs and cats follows similar clinical courses, including metastasis
Molecular basis of disease and molecular targets	Many of the genetic abnormalities (e.g. p53) and molecular therapeutic targets (e.g. receptor tyrosine kinases) are represented across the species.
Responses to therapy	For example the response to chemotherapy in NHL is similar in people as it is in dogs and cats.

4. Comparative telomere and telomerase biology

Hayflick and Moorhead originally observed that normal somatic cells have limited proliferative capacity and cannot grow indefinitely in culture [23]. After a finite number of divisions, cells reach their proliferative limit and enter “replicative senescence”. These cells are phenotypically characterised by a large, flattened morphology and expression of the biomarker “senescence associated β -galactosidase” [24]. Senescent cells are growth arrested in G1 phase of the cell cycle, are incapable of synthesising DNA, and are unresponsive to growth factors, yet are still metabolically active [10,11]. Subsequent studies have shown that telomeres become progressively shorter with each population doubling until they reach a critically short length and induce replicative senescence [25]. Consequently, telomeres have been eloquently described as “molecular clocks” that determines the life span of a cell [26].

Telomeres are specialised nucleoprotein complexes that cap and protect the end of every eukaryotic chromosome against chromosomal fusion, recombination, and terminal DNA degradation. Telomeres are composed of non-coding TTAGGG double strand repeats, orientated 5′-to-3′ toward the chromosome and ending in a 3′ single-stranded G-rich overhang [27]. To prevent degradation by exonucleases or recognition by the DNA damage machinery, the 3′ single-stranded overhang of telomeric DNA folds back into the D-loop of duplex telomeric DNA to form a protective T-loop, which is reinforced with telomere binding proteins [28,29]. In mammalian cells, telomeres associate with the shelterin complex of six core proteins: TRF1 (telomeric repeat-binding protein 1, also known as TERF1), TRF2 (also known as TERF2), TIN2 (TRF1-interacting nuclear factor 2), POT1 (protection of telomeres 1), TPP1 (POT-1- and TIN2-interacting protein), and RAP1 (transcriptional repressor/activator protein) [28,30] (Fig. 2). Telomeres that can no longer protect the end of chromosomes are said to be dysfunctional, and can arise by several mechanisms including progressive telomere attrition, loss of telomere binding proteins and direct telomeric DNA damage [31]. Dysfunctional telomeres can elicit potent DNA damage response pathways [12].

The length of telomeres in somatic cells can vary remarkably among individuals according to age, organ and proliferative history of each cell, for example telomere length in human somatic cells ranges from 5 to 20 kb [32]. During DNA synthesis and cell division, telomeres shorten due to the inability of DNA polymerase to copy the extreme 5′

ends of chromosomes, termed the ‘end replication problem’ [33]. Progressive telomere shortening represents a tumour suppressor mechanism and is proposed to be one of the molecular mechanisms underlying ageing, as critically short telomeres triggers replicative senescence and loss of cell viability [32,34]. Although most somatic cells have a finite number of cell divisions, some cells have evolved mechanisms to circumvent telomeric attrition therefore allowing continued proliferation [8,35]. The enzyme telomerase is able to maintain telomeres by synthesising telomeric DNA. Telomerase is a ribonucleoprotein complex consisting of an RNA template (TERC), which contains a template region complementary to the telomeric repeat sequence TTAGGG, and a reverse transcriptase catalytic subunit (TERT). The telomerase holoenzyme binds to the telomere through alignment of the RNA template region with the telomere repeat sequence. The protein component acts as a reverse transcriptase and catalyses the addition of telomeric repeats onto the ends of chromosomes using the RNA subunit as a template [36–38]. Telomerase activity prevents replication-dependent loss of telomeres and permits cells to overcome mammalian cell mortality. In agreement, ectopic expression of human telomerase is sufficient to stabilise telomeres in normal human fibroblast cells, bypass replicative senescence and allow immortal cell proliferation [39,40].

In humans, telomerase activity is tightly regulated by expression of the reverse transcriptase catalytic subunit (TERT). Telomerase is essential for embryogenesis but is repressed upon tissue differentiation during development and is absent from birth in most somatic tissues [41]. Cell populations that continue dividing throughout life, such as germ and stem cells, continue to express telomerase at reduced levels to maintain self-renewal capacity. In fact, low levels of telomerase activity have been found in some human adult stem cell compartments, including haematopoietic stem cells (HSCs) [42–44] and intestinal stem and progenitor cells in basal crypts [45]. Transient upregulation of telomerase activity has been observed in committed haematopoietic progenitor cells with increased cellular proliferation, possibly to reduce the rate of telomere loss during periods of rapid cell division and prevent premature telomere shortening. In more mature cells, telomerase activity is repressed independently of proliferation rate (Fig. 3) [46]. Significantly, a majority of human cancer cell lines and 85% of human cancers, encompassing a broad range of cancer types, possess telomerase activity. By contrast, little or no telomerase activity was detected in normal somatic tissues [47,48]. The expression of telomerase in a wide range of human cancers signifies the molecular mechanism by which most cancer cells gain immortality and represents an exciting therapeutic target.

Reactivation of telomerase is an imperative step in immortalisation of cancer cells and tumour progression. Currently the most commonly used *in vivo* model used to study telomeres and telomerase activity is the mouse. In mice, telomerase is present in all adult tissues and telomere lengths are much longer relative to humans, ranging from 40 to 80 kb, which are commonly reduced to a humanised length prior to experimentation by successive mTerc^{−/−} intercrossing [49,50]. In dogs, like humans, telomerase activity is upregulated in a majority of cancers and is absent in normal adult tissues [8]. The canine *Tert* promoter is similar to human promoter in structure and activity, and there is good correlation between *Tert* expression and telomerase activity in dog tissues [51,52]. Whilst the number of studies examining canine tumours is relatively small, it is evident that telomerase is present in the majority (>90%) of canine cancers and is absent from most normal tissues [8,51,53–55].

Importantly, telomere lengths are comparable in size to humans, and ageing canine fibroblast cells undergo telomere attrition in culture [51]. Human telomere and telomerase dynamics therefore appear to be conserved in dogs (Table 2). Currently, very little is known about the telomere length in canine tumours *per se* compared to normal tissues as such studies are lacking. In general, human tumour cells have shorter telomere lengths than normal cells and

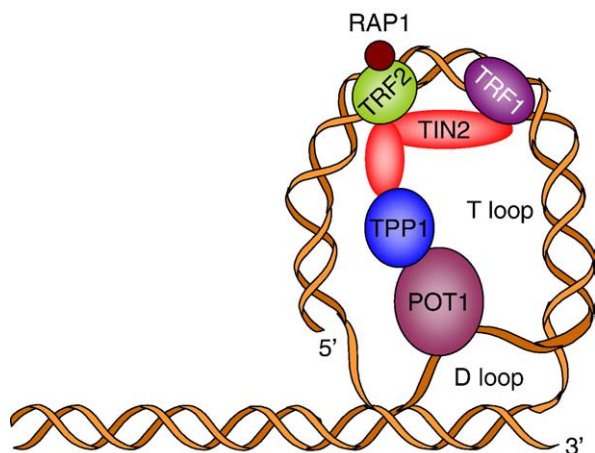


Fig. 2. Telomere structure. Telomeres are composed of TTAGGG repetitive sequences that terminate in a 3′ single-stranded overhang, which can invade the double stranded region of the telomere and form a protective T-loop with a single-stranded D-loop at the invasion site. Telomeric DNA is complexed with the shelterin protein complex composed of TRF1, TRF2, RAP1, TIN2, TPP1 and POT1. TPP1 and POT1 regulate the access of telomerase to the telomeric subunit.

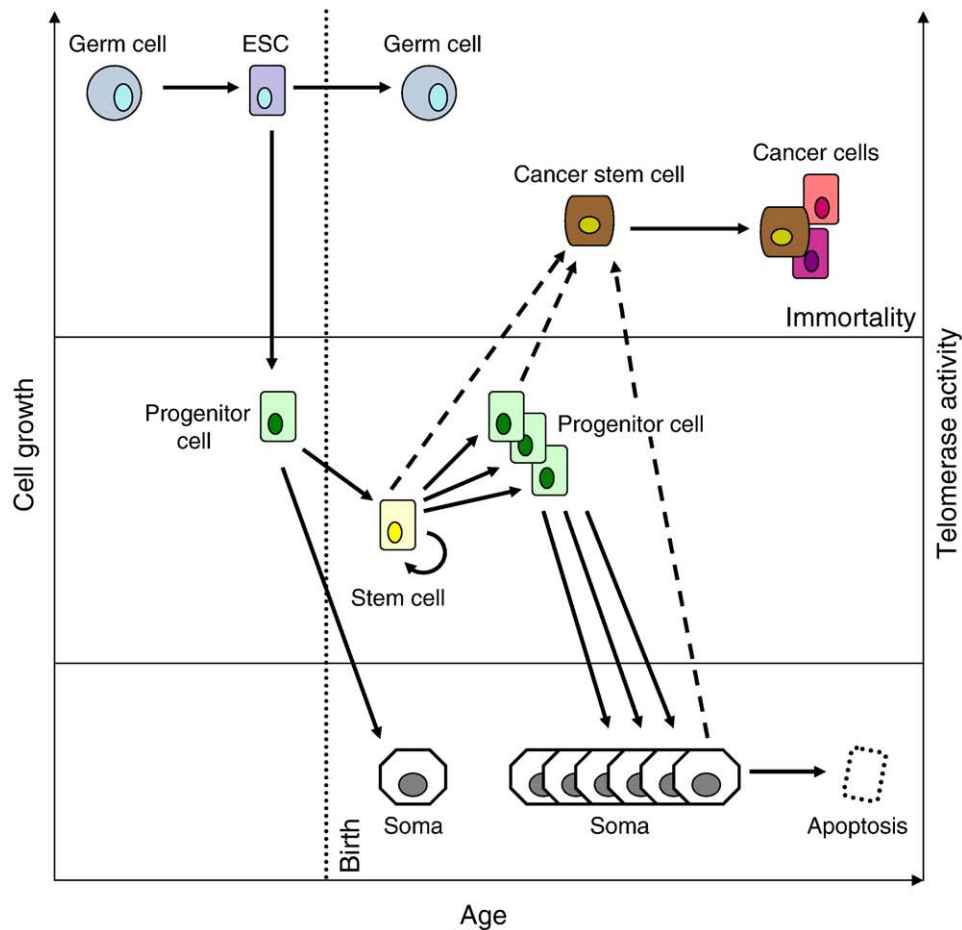


Fig. 3. Telomerase activity in relation to cell growth and age. Germ cells and embryonic stem cells (ESC) have high levels of telomerase activity. In the developmental stage, telomerase activity decreases and diminishes in most somatic cells after birth. Adult stem cells have a low level of telomerase that is upregulated in highly proliferative progenitor cells. However this is insufficient to maintain their telomere length, rendering these cells mortal and prone to telomere shortening. Cancer stem cells may be derived from adult stem, progenitor or somatic cells and may be immortal, having the ability to indefinitely self-renew and proliferate (adapted from Hiyama and Hiyama [13]).

show no net change in average telomere length with successive cell divisions, due to telomere maintenance by the enzyme telomerase. Some human tumour types (e.g. osteosarcomas [56], gliomas [57], soft tissue sarcomas and astrocytomas [58]) have been shown to maintain their telomeres by an alternative mechanism which has been termed alternative lengthening of telomeres or ALT and proposed to occur via homologous recombination. At present there is no conclusive evidence for ALT in canine cancers although a recent report suggests that this may occur in canine osteosarcoma [59].

5. Dysfunctional telomeres and tumorigenesis

As dysfunctional telomeres have lost their protective structure, either through telomere attrition or disruption of telomeric binding

proteins, they are recognised as damaged DNA and activate a DNA damage response pathway [60] (Fig. 4). In mammals, uncapped telomeres activate signalling cascades involving the protein kinases ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad-3 related). These kinases are recruited to telomeres and are activated, leading to the phosphorylation of histone H2AX adjacent to the site of DNA damage. Phosphorylated H2AX facilitates the focal assembly of checkpoint and DNA repair factors, including 53BP1 (p53 binding protein 1), NBS1 (also known nibrin) and MDC1. The DNA damaged foci around uncapped telomeres also promotes the ATM/ATR dependent phosphorylation and activation of the transducer kinases CHK2 and CHK1, which converge the signal on p53 [12,60]. The tumour suppressor protein p53 had been heralded as the “guardian of the genome” for its role in preserving genome integrity by regulating the expression of genes involved in growth arrest, DNA repair and apoptosis in response to DNA damage [61,62].

In the context of replicative senescence, p53 induces cell cycle arrest by stimulating expression of the cyclin-dependent kinase inhibitor p21^{WAF1} [63,64]. In the G1 phase of the cell cycle the RB (retinoblastoma) protein is hypophosphorylated, and in this state binds to and sequesters the E2F family of transcription factors, which promote S phase. Release of active E2F, which then leads to the transcription of genes required for S phase progression, is mediated by sequential phosphorylation of RB by cyclin D/CDK4 and cyclin E/CDK2 [65,66]. p21^{WAF1} inhibits cyclin E/CDK2, maintaining the RB-E2F complex, consequently preventing S phase entry and sustaining a G1 arrest [67,68]. Mutation of the p53 gene is the most tumour-related

Table 2
Comparative telomere and telomerase biology

Species	Telomere length	Telomerase activity
Mouse	>60 kb	Ubiquitous
Human	15–20 kb	Tumour tissue Stem cells Gonadal tissue Activated white blood cells
Dog	15–20 kb	Tumour tissue Stem cells Gonadal tissue Activated white blood cells

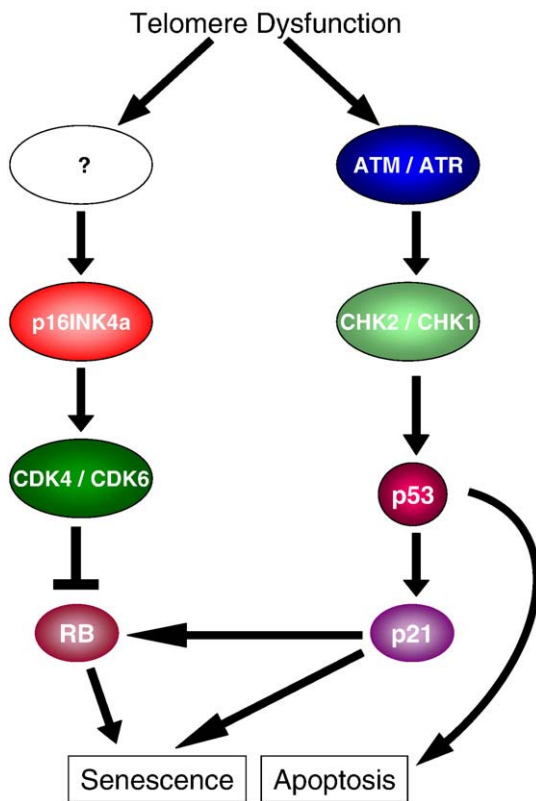


Fig. 4. Telomere dysfunction activates the p53 and RB pathways. Progressive telomere shortening or uncapped telomeres initiate a DNA damage response. Resulting in activation of ATM and ATR, and the downstream kinases CHK1 and CHK2, and phosphorylation of p53. Activated p53 inhibits tumourigenesis by transcriptionally upregulating genes that mediate cellular senescence and/or apoptosis. The p16INK4a/RB pathway inhibits cellular proliferation and is also activated by telomere dysfunction.

genetic abnormality, and is found in more than 50% of all human cancers [69]. The role of p53 in canine and feline tumours has also been extensively studied, and probably plays a major role in carcinogenesis in these species [70,71]. Functional inactivation of p53 in response to telomere dysfunction would provide a passive environment that tolerates proliferation and survival of genomically unstable cells, and may aid cancer progression. p16^{INK4a} is also a cyclin-dependent kinase inhibitor that maintains RB in a hypophosphorylated state [72], is markedly increased in senescent cells, and has been implicated in maintaining the senescence state [72,73]. When these cell cycle checkpoint proteins are inactivated by antisense oligonucleotides [74] or viral oncoproteins [75], cells bypass senescence and continue to proliferate. This leads to high cellular levels of genomic instability and induces p53-independent induction of cell death [76]. Few cells escape from this crisis period and those that do acquire the ability to stabilise telomeres, mainly through the reactivation of telomerase [40,77].

Uncapped telomeres are highly recombinogenic and can lead to improper chromosomal fusions [12,78]. During mitosis of the cell cycle the fused chromosomes will be separated and broken by the anaphase bridge, generating new telomere free chromosomal ends which will fuse and break again during the next round of cell division [79,80]. Hence telomere dysfunction can initiate a cascade of chromosomal gains, losses and translocations, creating novel chromosomal variants with each cell division [81,82]. Chromosomal instability is characteristic of tumourigenesis [81]. In human cancer, telomere shortening has been observed in very early tumour stages, indicating that tumours arise from cells with telomere dysfunction [83]. To evaluate the contribution of telomere dysfunction to tumourigenesis, telomerase deficient (mTerc^{-/-}) mice, which lack

the RNA subunit Terc, were generated [78]. Studies in mTerc^{-/-} mice showed that telomere shortening enhanced tumour initiation but suppressed tumour progression [80,84,85]. This is not surprising considering that telomere dysfunction induces the DNA damage pathways leading to senescence. In vitro experiments have shown that deletion of p53, in contrast to p16^{INK4a} deletion, can extend the life span of cells with critically short telomeres by bypassing senescence and increase susceptibility to oncogenic transformation by Myc and Hras [60]. In vivo, mTerc^{-/-}, p53^{-/-} mice do not have an extended life span compared to mTerc^{-/-} mice due to early tumour development. These tumours exhibit high rates of chromosomal instability [86,87]. Interestingly, the tumour spectrum of mTerc^{-/-}, p53^{+/+} mice resembled the high incidence of epithelial cancer in ageing humans [86], signifying that loss of p53 co-operates with telomere dysfunction to induce chromosomal instability and tumourigenesis.

6. Telomeres, telomerase and stem cells

Embryonic stem cells (ESC) are capable of indefinite self-renewal, can differentiate and contribute to the germ line. Experiments in both mouse ESCs [88] and human ESCs [89] have shown that these cells are unique in that they can proliferate indefinitely in culture, do not undergo replicative senescence and remain untransformed over multiple passages. In contrast to malignant tumour cells, ESCs respond to growth stimulation and inhibition signals, have a normal karyotype, and are able to differentiate into non-immortal cells that undergo telomere shortening and replicative senescence [90]. ESCs are inherently immortal, which is lost upon differentiation into somatic cells, and express high levels of TERT and correspondingly display high levels of telomerase activity. During differentiation down regulation of telomerase activity has been correlated with histone deacetylation and DNA methylation of the TERT gene [91]. Interestingly, cell cycle regulatory pathways in ESCs may significantly differ from those in differentiated somatic cells, as mouse ESCs were shown to have a defective Rb pathway and a nonresponsive p53 pathway [92–94]. The p53-dependent checkpoint was compromised as p53 was sequestered in the cytoplasm and unable to induce the transcription of genes necessary to induce cell cycle arrest, significantly the p53 pathway was restored after differentiation [92].

Although telomerase activity is very high during embryonic development this is downregulated in a majority of adult tissues after birth, with the exception of the adult stem cell compartments and cells that undergo rapid expansion, such as lymphocytes or skin keratinocytes [41] (Fig. 3). Adult stem cell compartments are important in the homeostatic maintenance of many organs, including organs with lower turn-over rates such as the brain and pancreatic islets. Stem cells in several tissues are maintained mainly in a quiescent state and undergo low but constant rates of cell turnover during their lifetime, which may require telomerase expression to prevent telomere shortening [4]. However, adult stem cells only maintain low levels of telomerase activity and this is not sufficient to maintain telomere length during stem cell ageing [95]. Although the presence of telomerase in these cells suggests that telomerase is important for stem cell function and organism fitness. Evidence that telomere shortening of stem cell compartments plays a causative role in ageing comes from the study of human diseases associated with mutations in the telomerase core components. Mutations in the TERC gene have been associated with the rare genetic disorders dyskeratosis congenita [96] and aplastic anaemia [97]. These diseases are characterised by premature loss of tissue regeneration due to loss of telomerase activity and telomere shortening, stem cell exhaustion, bone marrow failure and premature death [96,97]. Studies in Terc^{-/-} mice have shown that telomere shortening reduces stem cell function and is associated with impaired organ homeostasis of high turn-over organs, reduced life span, impaired organ regeneration and

impaired stress responses during ageing [98–101]. However over-expression of Tert in transgenic mouse models results in increased tumorigenesis [102–104], signifying that ageing maybe the evolutionary consequence of cancer protection (Fig. 5).

Telomerase activity has been implicated in both cancer and ageing. These important biological processes have been proposed to share common aspects of biology, in that they are both stem cell diseases resulting from a defect or decline in regenerative capacity and organ homeostasis. DNA damage may accumulate in self-renewing stem cell compartments with increasing age until normal stem cell biology is perturbed. Tumour suppressor mechanisms are activated to remove damaged cells by either apoptosis or senescence. Apoptosis of stem cells may have less severe effects on regenerative potential of an organ than senescence. For example, apoptotic cells can be cleared from the stem cell niche, allowing viable stem cells to self-renew, replace damaged cells and facilitate regeneration. However senescent cells continue to occupy the niche and prevent replenishment of the stem cell compartment. Therefore senescence may enhance stem cell attrition and the appearance of age-related phenotypes. If oncogenic mutations accumulate, self-renewing clones that contain such lesions might undergo positive selection, leading to cancer (Fig. 6). Hence, cancer and ageing maybe related endpoints of DNA damage accumulation in adult stem cell compartments.

7. Cancer stem cells

The cancer stem cell theory has challenged the accepted paradigm of tumorigenesis, where any cell in the body has the potential for malignant transformation. The clonal evolution model of cancer postulates that mutant tumour cells with a growth advantage are selected and expanded, with all cells in the resulting tumour having similar potential for regenerating tumour growth (Fig. 7A, B) [105]. In conflict with this model, the bulk of cells in an organ have a finite life span dictated by telomere attrition [106], therefore how does a cell survive long enough to acquire the number of mutations required for malignant transformation? Also the majority of therapeutic approaches aimed at eliminating tumour cells are based on the clonal evolution model. The limited effects of these conventional therapies suggest that tumour cells include a resistant population of cells responsible for tumour initiation, development, growth and reoccurrence. The discovery of a rare subset of cells with stem cell qualities in acute myeloid leukaemia, that could induce leukaemia when transplanted into immunodeficient mice, has revolutionised the way tumorigenesis is viewed and formed the foundation of the cancer

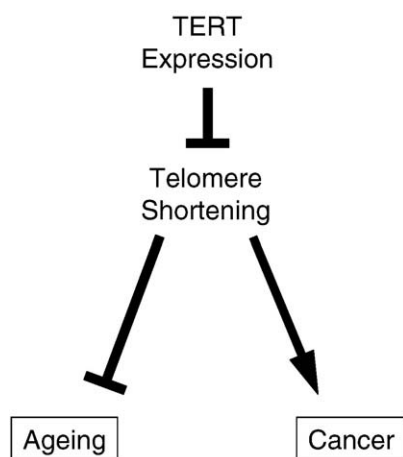


Fig. 5. Telomerase expression effects ageing and cancer. In the Tert transgenic mouse model where Tert is overexpressed, the ageing phenotype is suppressed but there is an increase in tumorigenesis.

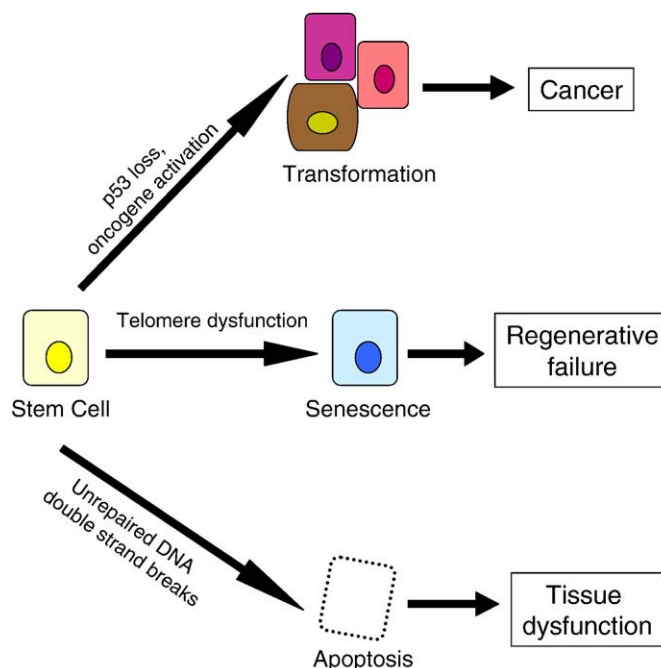


Fig. 6. The fate of stem cells with heritable DNA damage. Different DNA lesions are associated with each outcome as indicated.

stem cell model [107]. In the cancer stem cell model, tumour development, like the normal development of a tissue, relies upon a subset of tumour cells that have ability to self-renew and generate the diverse cells that comprise the tumour (Fig. 7C) [5]. Only those cells within the tumour that can self-renew and differentiate are called cancer stem cells. Therefore cancer stem cells can expand the cancer stem cell pool and differentiate into cancer progenitor cells by symmetric and asymmetric division, respectively. Consequently, daughter cells lacking stem cell qualities will have only limited proliferative potential but constitute the bulk of a tumour mass. In this model, a tumour can be viewed as a heterogeneous and regenerative tissue, where cancer stem cells are responsible for initiation and maintenance of the cancer. This provides an explanation for traditional chemo- and radiotherapies which often shrink the tumour bulk but do not completely eradicate it, indicating that conventional therapies cannot effectively target cancer stem cells embedded within a given tumour mass.

The first evidence for the existence of cancer stem cells came from acute myeloid leukaemia. Bonnet and Dick isolated a rare subpopulation of cells with the surface marker expression pattern of CD34+/CD38–, characteristic of haematopoietic stem cells, from patients with acute myeloid leukaemia [107]. Despite being small in numbers (0.2% of the tumour population), these were the only cells that could induce leukaemia phenotypically identical to the parent tumour after transplanting them into non-obese diabetic severe combined immunogenicity (NOD-SCID) mice. Cells with the typical leukaemia phenotype CD34+/CD38+ were not capable of initiating tumour development. From these observations and the hierarchical cell profile of leukaemia, reminiscent of haematopoiesis, Bonnet and Dick concluded that malignant transformation occurs in the haematopoietic stem cell and gives rise to leukaemic stem cells [107].

More recently cancer stem cells have been demonstrated in solid tumours. The first solid tumour from which cancer stem cells were identified and isolated was breast cancer. Al-Hajj et al. [108] described a CD44+/CD24– cell population that was significantly enriched for tumour initiation. Human breast tumour samples were found to be heterogeneous in surface antigen expression, when different populations were injected into NOD-SCID mice, only cells

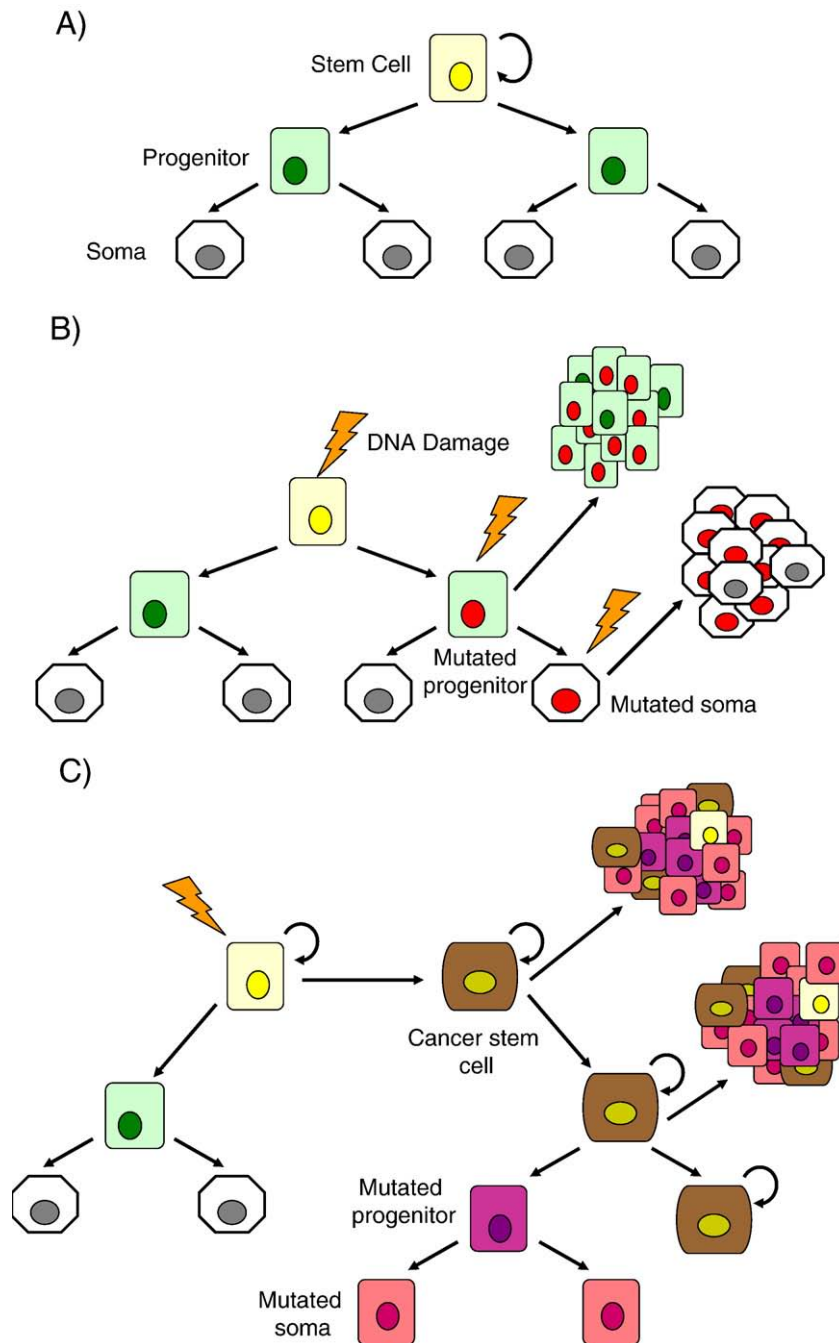


Fig. 7. Models of tumour formation. (A) A normal cellular hierarchy comprising of adult stem cells at the apex, which generate progenitor cells, ultimately yielding all mature cell types that constitute that particular tissue. (B) In the clonal evolution model all cells have the same tumorigenic capacity and any cell can initiate and maintain a tumour. (C) In the cancer stem cell model, only cancer stem cells can generate a tumour due to its self-renewal and proliferative capacity. Cancer stem cells can also give rise to mutated progenitor and mature cells that constitute the bulk of the tumour, and account for tumour heterogeneity.

expressing CD44+/CD24– form tumours. The resulting tumours contained multiple cell types, similar to the original tumour, indicating that CD44+/CD24– cells were not only capable of self-renewal but also of generating a range of progeny [108]. Subsequently, CD133 was found to mark cancer stem cells in different types of brain tumours including glioblastoma, medulloblastoma, and ependymomas [109–111]. CD133 is a cell-surface marker previously shown to be expressed on neural stem cells [112]. However, CD133 has also been utilized to isolate and identify cancer stem cells in colorectal [113,114] and pancreatic carcinomas [115], and has been implicated as a common stemness marker in cancer stem cells with different origins [116]. Cancer stem cells have also been identified in

bladder [117], head and neck squamous cell carcinomas [118], bone sarcomas [119], melanomas [120], retinoblastomas [121], prostate [122], hepatic [123] and lung tumours [124].

The exact origins of cancer stem cells may vary. They could arise from the malignant transformation of a normal stem cell that has accumulated oncogenic mutations over time. Many of the attributes of normal stem cells make them attractive candidates for malignant transformation into cancer stem cells, they are programmed for self-renewal and differentiation, and they persist and continue to divide for the lifetime of the host, allowing them opportunity to amass oncogenic lesions that lead to transformation. In support of this model, isolated cancer stem cells share the same cell-surface marker

expression pattern as the corresponding normal tissue stem cell [106]. Alternatively, the original tumour cell could be a more differentiated cell that has acquired stem cell characteristics by mutation and dedifferentiation [125]. The recent demonstration that adult fibroblasts can be reprogrammed into pluripotent ES-like cells by expression of four genes Oct3/4, Sox2, c-Myc, and Klf4 [126], raises the possibility that expression of these stem cell factors and specific oncogenes could induce a dedifferentiated state in cancer cells. These factors and additional ESC specific genes have been shown to be highly expressed in poorly differentiated breast, glioblastoma and bladder carcinomas [127], suggesting that cancer cells may utilize the newly identified “iPS reprogramming” mechanism [126] to obtain stem cell characteristics, bypass senescence and gain immortality.

The ability of a cancer stem cell to initiate and drive tumourigenesis may be influenced by its niche environment, which is important for normal stem cell function. The stem cell niche is an anatomical compartment that provides signals to stem cells, via secreted and cell-surface molecules, to control the rate of stem cell proliferation, determine the fate of daughter cells, and protect stem cells from exhaustion and death [128]. There is compelling evidence that the tumour microenvironment promotes and sustains abnormal cell growth of cancer cells. In the brain, both normal neural stem cells and cancer stem cells are found in the region with a high capillary

density, and the main component of these niches is endothelial cells. Transplantation human medullablastoma cells with or without the surrounding endothelial cells into NOD-SCID mice both caused cancer, but co-transfection with the niche cells enhanced the tumour size and growth rate [129]. Furthermore, expression profiling of stromal cells that are associated with human basal cell carcinomas demonstrated that tumour associated stroma, but not stroma associated with normal skin, expresses secreted factors that block the differentiation of epithelial cells within the tumour [130]. These studies highlight an intricate relationship between the niche and cancer stem cells.

8. The canine and feline model systems

Mechanisms of tumour development appear to be conserved between human and dogs. Canine “cancer stem cells” have been successfully isolated from osteosarcomas and have been shown to express embryonic stem cell markers Oct 3/4 and Nanog, which are involved maintenance of pluripotency [131]. Similar cancer stem cell populations have been isolated from mammary carcinoma, melanoma, and haemangiosarcoma (unpublished results). In addition, feline mammary tumour and squamous cell carcinoma initiating cells have been identified from cell lines and clinical cases [132]. These cells

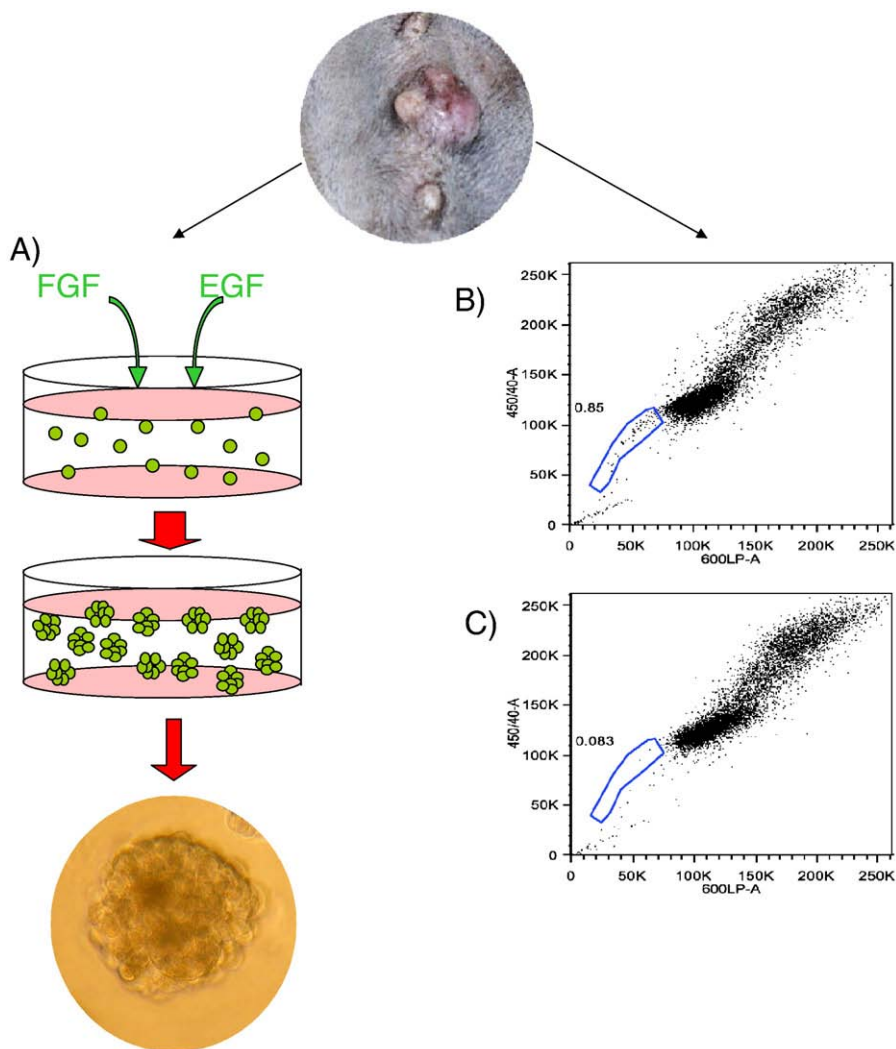


Fig. 8. Isolation of tumour initiating cells using the classical sphere assay and FACS analysis. (A) Single cells from cell lines and clinical cases are cultured in non-adherent culture conditions using serum-starved semi-solid media. Spheres form in ~7–10 days and express embryonic stem cell markers including Oct4 and Nanog. (B) FACS can be used to identify a small “side” population of cells that have an enhanced ability to exclude dyes, such as Hoechst 33342, potentially through expression of the ATP binding cassette protein transporters. (C) The addition of Verapamil demonstrates loss of the side population by drug inhibition of efflux of Hoechst 33342.

have been isolated using both the classical “sphere” assay and utilizing FACS analysis (Fig. 8) and interestingly have demonstrated reduced sensitivities to both chemotherapy and radiation [132]. On a practical level, there are many advantages of utilizing dogs and cats in the study of cancer stem cell biology:

- Access to biopsy material from clinical naive animals allows interrogation of the cancer stem cell theory in the natural setting.
- Access to post-mortem material allows for close monitoring and follow-up of clinical cases.
- Dogs and cats represent a pre-clinical model system to assess therapeutics targeted to cancer stem cells.

9. Integrating canine and feline models to develop new therapies

With the evolution of the cancer stem cell theory, investigators have focused on potential therapeutic targets including signalling pathways and mechanisms that contribute to drug resistance (Table 3 and Refs. [133–175]). Molecular mechanisms that regulate critical functions in normal stem cells, including self-renewal and proliferation, have been shown to be deregulated in human cancers. The tumour suppressor proteins that inhibit tumour proliferation or regulate cellular responses to DNA damage, such as p53, PTEN, p16^{INK4a} and ARF, may also block stem cell self-renewal [106].

In preceding sections we have described the biological similarities between cancers in humans and companion animals (dogs and cats). In addition to providing a new model system to study cancer stem cell biology, the biological similarities between the species may predict

Table 3
Potential therapeutic targets based upon cancer stem cells and telomerase

Therapeutic strategy	Comment
Improving radiosensitivity	Inhibition of the checkpoint kinases CHK1 and CHK2 may reduce radioresistance of CD133+ cells to levels comparable to CD133– cells.
Signalling pathways	Hedgehog, Notch and Wnt/ β -catenin pathways regulate stem cell self-renewal and may offer therapeutic targets. Inhibition of Hedgehog–Gli1 with cyclopamine in mice with highly aggressive glioblastoma led to a reduced proliferation of CD133+ cells and prolonged survival of the animals [148]. Cyclopamine was also able to cause tumour reduction in mouse models of pancreas and prostate cancer [149,150]. Targeted disruption of the Notch pathway by inhibition of γ -secretase with GSI-18 led to a reduction of CD133+ cells, slower tumour growth and apoptosis in the medulloblastoma, in vitro [151]. Wnt/ β -catenin signalling was shown to be essential for sustaining skin cancer stem cells but not normal epidermal stem cells, this distinction may enable specific targeting of cancer stem cells in squamous cell carcinoma [127].
Targeting the stem cell niche	However a combination of anti-angiogenic therapy and chemotherapy may deregulate the niche and sensitise cells to chemotherapy.
Telomerase targeting	GRN163L is a modified oligonucleotide complementary to TERC and is currently in clinical trials [154]. G-quadruplex inducing agents, including RHP54 and BRACO19 [158,159]. The G-rich strand of telomeric DNA can fold into a four stranded G-quadruplex, stabilisation of which perturbs telomere function. As TERT is expressed in the majority of human cancers, it has been identified as a general tumour antigen. Promoter directed therapy offers an elegant system by which gene expression can be targeted to cells, which the promoter is switched on. Recently, telomerase-dependent conditionally replicative adenoviral vectors have been developed [167].

Cancer stem cells are thought to have a greater resistance to either chemotherapy (through the expression of active transport mechanisms or expression of anti-apoptotic proteins) or radiotherapy (through enhanced DNA damage response pathways). This table illustrates some potential alternative approaches.

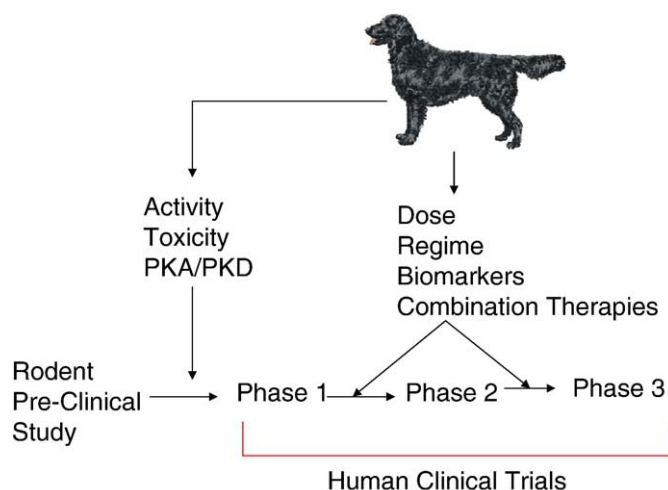


Fig. 9. The integration of canine studies into human clinical drug development (adapted from Paoloni and Khanna [176]).

similarities in treatment responses between canine, feline and human cancers [176]. The continued evolution and validation of the cancer stem cell model demands re-evaluation of conventional cancer therapies. Chemo- and radiotherapy are based on the assumption that all cells within a tumour are the same, and although these approaches do significantly reduce the tumour mass, progression and/or relapse do occur and could be due to the persistence of resistant cancer stem cells. A two-pronged strategy may be required to remove both tumour initiating cells and tumour maintaining cells. To develop such therapies the molecular mechanisms of resistance must be established and circumvented, and novel pathways in cancer stem cells must be evaluated. It is quite possible that the study of cancer stem cell biology and potential therapeutic strategies in dogs and cats may facilitate the rapid translation into human clinical trials.

Classical drug development follows a linear pathway from mouse to phase 1, 2 and 3 clinical trials in human subjects. However, many drugs that show efficacy in mouse models often fail in human clinical trials because of either a lack of efficacy or unacceptable toxicity [15]. This highlights the potential problems of a reliance on rodent models and makes drug development costly and inefficient. The natural canine or feline models may overcome some of these shortcomings because their tumours demonstrate similar genomic instability and heterogeneity to human cancers and may also allow key biological

Table 4
The potential disadvantages of utilizing natural models

Issue	Comment
Species differences Cancer types	There may be species variation in drug sensitivity. Although most human cancer types are represented in the dog, the dog tends to get more mesenchymal and lymphoid tumours. The incidence of epithelial cancers is lower in dogs and trials may take longer to conduct.
Regulatory issues	There is variation in this between Europe and the USA, but studies in dogs and cats are closely regulated by individual governments and all studies require ethical and scientific approval.
Inbred vs. outbred	It has been suggested that the “inbred” nature of dogs does not lend itself well to cancer studies reflecting an outbred population. In fact the germline genetic diversity of a population of dogs with a given cancer is similar to the diversity seen in well mixed human populations.
Cost	It is more expensive to conduct trials in dogs and cats than in mice. However, considering the potential of drug failure going directly from mouse studies into human studies, the use of dogs and cats could potentially save the pharmaceutical industry many millions of dollars.

questions to be answered that would be difficult in either rodent or human models alone.

There are several examples where dogs have provided key insights into cancer biology and therapy [92,177–179]. In a recent paper by Paoloni and Kahnna [176] the authors suggest that the dog model could be integrated into the current linear drug development model. In this approach, studies of novel compounds could be performed in pet dogs with cancer and better inform both early drug development parameters (activity, toxicity, pharmacokinetics and pharmacodynamics) and later parameters such as dose, schedule, biomarkers and the effects of combining drug strategies (Fig. 9). In the development of biologics or small molecules that target telomerase or cancer stem cell pathways, this could be an essential step to streamline drug development. The translational opportunities could be further broadened to go beyond observational studies and include:

- Detailed biological studies utilizing information from the canine genome project and canine-specific molecular tools.
- Utilization of study interventions that would be difficult to do in human oncology (e.g. serial biopsy samples).
- Identification of surrogate markers, or surrogate or biological endpoints.
- Utilization of advanced in vivo imaging technologies such as PET-CT.

Some of these concepts of comparative oncology have been adopted in the United States through the comparative oncology programme of the NCI, and a number of studies have already been performed utilizing canine models [176].

10. Future directions

The study of telomerase and cancer stem cell biology in both dogs and cats offers two opportunities. Firstly, cancer is common in these species and, as with human medicine, surgery, chemotherapy and radiation are still the only realistic treatment options. The identification of therapeutic targets in these species may inform better and safer drug development for dogs and cats. Secondly, parallel studies on cancer stem cell biology between relevant models (dog, cat, human) may offer an opportunity to integrate veterinary-based drug studies into human drug development. There is no doubt that the rodent model is very efficient and very useful, and the authors are not advocating exchanging this model for dogs and cats. There are equally some drawbacks of using natural models (Table 4). However, the drawbacks of rodent models need to be recognised and the potential of natural models should be explored as a complimentary approach. To develop therapies based upon a greater understanding of cancer biology, one must consider:

- Are rodent models appropriate to evaluate novel therapies?
- Are classical linear phase 1, 2 or 3 clinical trials in people appropriate to establish biological endpoints?
- Can studies on the naturally occurring cancers in dogs and cats answer questions that cannot be answered in human or murine studies alone?
- Would the integration of studies in cats and dogs into human clinical studies help to make drug development more efficient and less costly.

The answers to these points will vary depending on the cancer under study and the biological question being posed. However, the studies that have been performed through the NCI suggest that naturally occurring cancers in dogs provide us with a unique opportunity to advance cancer biology and therapy. Studies should continue to focus on specific biological questions and the availability of canine and feline tumour banks, alongside species-specific reagents should allow us to achieve this. The prospect of developing drugs which target key pathways in either telomerase biology or cancer

stem cells lends itself well to this and will allow better design of human clinical trials.

Acknowledgement

Work by the authors in domestic animal cancer stem cells is funded by the BBSRC.

References

- [1] K.W. Orford, D.T. Scadden, Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation, *Nat. Rev. Genet.* 9 (2) (2008) 115–128.
- [2] T.A. Rando, Stem cells, ageing and the quest for immortality, *Nature* 441 (7097) (2006) 1080–1086.
- [3] U.R. Rapp, F. Ceteci, R. Schreck, Oncogene-induced plasticity and cancer stem cells, *Cell Cycle* 7 (1) (2008) 45–51.
- [4] N.E. Sharpless, R.A. DePinho, How stem cells age and why this makes us grow old, *Nat. Rev. Mol. Cell. Biol.* 8 (9) (2007) 703–713.
- [5] R.W. Cho, M.F. Clarke, Recent advances in cancer stem cells, *Curr. Opin. Genet. Dev.* 18 (2008) 1–6.
- [6] B. Vogelstein, K.W. Kinzler, Cancer genes and the pathways they control, *Nat. Med.* 10 (2004) 789–799.
- [7] A.L. Fridman, M.A. Tainsky, Critical pathways in cellular senescence and immortalization revealed by gene expression profiling, *Oncogene* 27 (46) (2008) 5975–5987.
- [8] D.J. Argyle, L. Nasir, Telomerase: a potential diagnostic and therapeutic tool in canine oncology, *Vet. Pathol.* 40 (1) (2003) 1–7.
- [9] M.V. Blagosklonny, Cell immortality and hallmarks of cancer, *Cell Cycle* 2 (4) (2003) 296–299.
- [10] N.G. Berube, J.R. Smith, O.M. Pereira-Smith, The genetics of cellular senescence, *Am. J. Hum. Genet.* 62 (5) (1998) 1015–1019.
- [11] A.S. Lundberg, et al., Genes involved in senescence and immortalization, *Curr. Opin. Cell. Biol.* 12 (6) (2000) 705–709.
- [12] F. d'Adda di Fagagna, et al., A DNA damage checkpoint response in telomere-initiated senescence, *Nature* 426 (6963) (2003) 194–198.
- [13] E. Hiyama, K. Hiyama, Telomere and telomerase in stem cells, *Br. J. Cancer* 96 (7) (2007) 1020–1024.
- [14] J. Campisi, Cellular senescence as a tumor-suppressor mechanism, *Trends Cell. Biol.* 11 (11) (2001) S27–S31.
- [15] A. Kamb, S. Wei, C. Lengauer, Why is cancer drug discovery so difficult? *Nat. Rev. Drug. Discov.* 6 (2) (2007) 115–120.
- [16] C. Khanna, et al., The dog as a cancer model, *Nat. Biotechnol.* 24 (9) (2006) 1065–1066.
- [17] O.a.O.D. Jarrett, Leukaemogenic viruses, in: J.A. Whittaker (Ed.), *Leukaemia*, second edition, Published by Blackwells Scientific Publications, Oxford, 1992, pp. 34–63.
- [18] M.S. Campo, et al., Association of bovine papillomavirus type 2 and bracken fern with bladder cancer in cattle, *Cancer. Res.* 52 (24) (1992) 6898–6904.
- [19] D.J. Argyle, C. Khanna, The biology of cancer, in: S. Withrow, D. Vail (Eds.), *Small Animal Clinical Oncology*, Elsevier, 2006, pp. 31–53.
- [20] A. Porrello, P. Cardelli, E.P. Spugnini, Oncology of companion animals as a model for humans. An overview of tumor histotypes, *J. Exp. Clin. Cancer Res.* 25 (1) (2006) 97–105.
- [21] K. Lindblad-Toh, et al., Genome sequence, comparative analysis and haplotype structure of the domestic dog, *Nature* 438 (7069) (2005) 803–819.
- [22] R. Thomas, et al., Chromosome aberrations in canine multicentric lymphomas detected with comparative genomic hybridisation and a panel of single locus probes, *Br. J. Cancer* 89 (8) (2003) 1530–1537.
- [23] L. Hayflick, P.S. Moorhead, The serial cultivation of human diploid cell strains, *Exp. Cell. Res.* 25 (1961) 585–621.
- [24] G.P. Dimri, et al., A biomarker that identifies senescent human cells in culture and in aging skin in vivo, *Proc. Natl. Acad. Sci. U. S. A.* 92 (20) (1995) 9363–9367.
- [25] V.J. Cristofalo, R.J. Pignolo, Replicative senescence of human fibroblast-like cells in culture, *Physiol. Rev.* 73 (3) (1993) 617–638.
- [26] Y.S. Cong, W.E. Wright, J.W. Shay, Human telomerase and its regulation, *Microbiol. Mol. Biol. Rev.* 66 (3) (2002) 407–425 (table of contents).
- [27] E.H. Blackburn, Structure and function of telomeres, *Nature* 350 (6319) (1991) 569–573.
- [28] T. de Lange, Shelterin: the protein complex that shapes and safeguards human telomeres, *Genes Dev.* 19 (18) (2005) 2100–2110.
- [29] J.D. Griffith, et al., Mammalian telomeres end in a large duplex loop, *Cell* 97 (4) (1999) 503–514.
- [30] D. Liu, et al., Telosome, a mammalian telomere-associated complex formed by multiple telomeric proteins, *J. Biol. Chem.* 279 (49) (2004) 51338–51342.
- [31] P. Phatak, A.M. Burger, Telomerase and its potential for therapeutic intervention, *Br. J. Pharmacol.* 152 (7) (2007) 1003–1011.
- [32] W.E. Wright, J.W. Shay, Telomere biology in aging and cancer, *J. Am. Geriatr. Soc.* 53 (9 Suppl) (2005) S292–S294.
- [33] J.D. Watson, Origin of concatemeric T7 DNA, *Nat. New Biol.* 239 (94) (1972) 197–201.
- [34] J. Campisi, The biology of replicative senescence, *Eur. J. Cancer* 33 (5) (1997) 703–709.
- [35] L. Nasir, Telomeres and telomerase: biological and clinical importance in dogs, *Vet. J.* 175 (2) (2008) 155–163.

- [36] J. Feng, et al., The RNA component of human telomerase, *Science* 269 (5228) (1995) 1236–1241.
- [37] M. Meyerson, et al., hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization, *Cell* 90 (4) (1997) 785–795.
- [38] T.M. Nakamura, et al., Telomerase catalytic subunit homologs from fission yeast and human, *Science* 277 (5328) (1997) 955–959.
- [39] A.G. Bodnar, et al., Extension of life-span by introduction of telomerase into normal human cells, *Science* 279 (5349) (1998) 349–352.
- [40] C.M. Counter, et al., Stabilization of short telomeres and telomerase activity accompany immortalization of Epstein–Barr virus-transformed human B lymphocytes, *J. Virol.* 68 (5) (1994) 3410–3414.
- [41] W.E. Wright, et al., Telomerase activity in human germline and embryonic tissues and cells, *Dev. Genet.* 18 (2) (1996) 173–179.
- [42] D. Broccoli, J.W. Young, T. de Lange, Telomerase activity in normal and malignant hematopoietic cells, *Proc. Natl. Acad. Sci. U. S. A.* 92 (20) (1995) 9082–9086.
- [43] C.M. Counter, et al., Telomerase activity in normal leukocytes and in hematologic malignancies, *Blood* 85 (9) (1995) 2315–2320.
- [44] K. Hiyaama, et al., Activation of telomerase in human lymphocytes and hematopoietic progenitor cells, *J. Immunol.* 155 (8) (1995) 3711–3715.
- [45] W. Yasui, et al., Immunohistochemical detection of human telomerase reverse transcriptase in normal mucosa and precancerous lesions of the stomach, *Jpn. J. Cancer Res.* 90 (6) (1999) 589–595.
- [46] C.P. Chiu, et al., Differential expression of telomerase activity in hematopoietic progenitors from adult human bone marrow, *Stem. Cells* 14 (2) (1996) 239–248.
- [47] N.W. Kim, et al., Specific association of human telomerase activity with immortal cells and cancer, *Science* 266 (5193) (1994) 2011–2015.
- [48] J.W. Shay, S. Bacchetti, A survey of telomerase activity in human cancer, *Eur. J. Cancer* 33 (5) (1997) 787–791.
- [49] R.A. Greenberg, et al., Expression of mouse telomerase reverse transcriptase during development, differentiation and proliferation, *Oncogene* 16 (13) (1998) 1723–1730.
- [50] L. Martin-Rivera, et al., Expression of mouse telomerase catalytic subunit in embryos and adult tissues, *Proc. Natl. Acad. Sci. U. S. A.* 95 (18) (1998) 10471–10476.
- [51] L. Nasir, et al., Telomere lengths and telomerase activity in dog tissues: a potential model system to study human telomere and telomerase biology, *Neoplasia* 3 (4) (2001) 351–359.
- [52] S. Long, et al., The canine telomerase catalytic subunit (dogTERT): characterisation of the gene promoter and identification of proximal core sequences necessary for specific transcriptional activity in canine telomerase positive cell lines, *Gene* 358 (2005) 111–120.
- [53] Y. Funakoshi, et al., Cellular proliferative and telomerase activity in canine mammary gland tumors, *Vet. Pathol.* 37 (2) (2000) 177–183.
- [54] M. Yazawa, et al., Telomere length and telomerase activity in canine mammary gland tumors, *Am. J. Vet. Res.* 62 (10) (2001) 1539–1543.
- [55] M. Yazawa, et al., Measurement of telomerase activity in dog tumors, *J. Vet. Med. Sci.* 61 (10) (1999) 1125–1129.
- [56] G.A. Ulaner, et al., Absence of a telomere maintenance mechanism as a favorable prognostic factor in patients with osteosarcoma, *Cancer Res.* 63 (8) (2003) 1759–1763.
- [57] V. Hakin-Smith, et al., Alternative lengthening of telomeres and survival in patients with glioblastoma multiforme, *Lancet* 361 (9360) (2003) 836–838.
- [58] J.D. Henson, et al., A robust assay for alternative lengthening of telomeres in tumors shows the significance of alternative lengthening of telomeres in sarcomas and astrocytomas, *Clin. Cancer Res.* 11 (1) (2005) 217–225.
- [59] K. Kow, S.M. Bailey, E.S. Williams, S. Withrow, S.E. Lana, Telomerase activity in canine osteosarcoma, *Vet. Comp. Oncol.* 4 (3) (2006) 178–183.
- [60] Y. Deng, S.S. Chan, S. Chang, Telomere dysfunction and tumour suppression: the senescence connection, *Nat. Rev. Cancer* 8 (6) (2008) 450–458.
- [61] A.M. Bode, Z. Dong, Post-translational modification of p53 in tumorigenesis, *Nat. Rev. Can.* 4 (2004) 793–805.
- [62] D.P. Lane, Cancer: p53, guardian of the genome, *Nature* 358 (1992) 15–16.
- [63] V. Dulic, et al., p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest, *Cell* 76 (1994) 1013–1023.
- [64] W.S. el-Deiry, et al., WAF1, a potent mediator of p53 tumour suppression, *Cell* 75 (1993) 817–825.
- [65] H. Müller, et al., E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis, *Genes Dev.* 15 (2001) 267–285.
- [66] C.J. Sherr, G1 phase progression: cycling on cue, *Cell* 79 (1994) 551–555.
- [67] J.W. Harper, et al., The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases, *Cell* 75 (1993) 805–816.
- [68] J.W. Harper, et al., Inhibition of cyclin-dependent kinases by p21, *Mol. Biol. Cell* 6 (1995) 387–400.
- [69] A.J. Levine, p53, the cellular gatekeeper for growth and division, *Cell* 88 (1997) 323–331.
- [70] L. Nasir, et al., Immunocytochemical analysis of the tumour suppressor protein (p53) in feline neoplasia, *Cancer Lett.* 155 (1) (2000) 1–7.
- [71] L. Nasir, et al., Analysis of p53 mutational events and MDM2 amplification in canine soft-tissue sarcomas, *Cancer Lett.* 174 (1) (2001) 83–89.
- [72] W.Y. Kim, N.E. Sharpless, The regulation of INK4/ARF in cancer and aging, *Cell* 127 (2) (2006) 265–275.
- [73] J. Campisi, Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors, *Cell* 120 (4) (2005) 513–522.
- [74] E. Hara, et al., Cooperative effect of antisense-Rb and antisense-p53 oligomers on the extension of life span in human diploid fibroblasts, TIG-1, *Biochem. Biophys. Res. Commun.* 179 (1) (1991) 528–534.
- [75] J.W. Shay, O.M. Pereira-Smith, W.E. Wright, A role for both RB and p53 in the regulation of human cellular senescence, *Exp. Cell Res.* 196 (1) (1991) 33–39.
- [76] A. Lechel, et al., The cellular level of telomere dysfunction determines induction of senescence or apoptosis in vivo, *EMBO Rep.* 6 (3) (2005) 275–281.
- [77] C.M. Counter, et al., Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity, *Embo J.* 11 (5) (1992) 1921–1929.
- [78] M.A. Blasco, et al., Telomere shortening and tumor formation by mouse cells lacking telomerase RNA, *Cell* 91 (1) (1997) 25–34.
- [79] K.E. Kirk, et al., Block in anaphase chromosome separation caused by a telomerase template mutation, *Science* 275 (5305) (1997) 1478–1481.
- [80] K.L. Rudolph, et al., Telomere dysfunction and evolution of intestinal carcinoma in mice and humans, *Nat. Genet.* 28 (2) (2001) 155–159.
- [81] C. Lengauer, K.W. Kinzler, B. Vogelstein, Genetic instabilities in human cancers, *Nature* 396 (6712) (1998) 643–649.
- [82] R.S. Maser, R.A. DePinho, Connecting chromosomes, crisis, and cancer, *Science* 297 (5581) (2002) 565–569.
- [83] R.R. Plentz, et al., Telomere shortening of epithelial cells characterises the adenoma-carcinoma transition of human colorectal cancer, *Gut* 52 (9) (2003) 1304–1307.
- [84] E. Gonzalez-Suarez, et al., Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis, *Nat. Genet.* 26 (1) (2000) 114–117.
- [85] R.A. Greenberg, et al., Short dysfunctional telomeres impair tumorigenesis in the INK4a(delta2/3) cancer-prone mouse, *Cell* 97 (4) (1999) 515–525.
- [86] S.E. Artandi, et al., Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice, *Nature* 406 (6796) (2000) 641–645.
- [87] L. Chin, et al., p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis, *Cell* 97 (4) (1999) 527–538.
- [88] M.J. Evans, M.H. Kaufman, Establishment in culture of pluripotent cells from mouse embryos, *Nature* 292 (5819) (1981) 154–156.
- [89] J.A. Thomson, et al., Embryonic stem cell lines derived from human blastocysts, *Science* 282 (5391) (1998) 1145–1147.
- [90] X. Zeng, Human embryonic stem cells: mechanisms to escape replicative senescence? *Stem. Cell. Rev.* 3 (4) (2007) 270–279.
- [91] N.G. Lopatina, et al., Control mechanisms in the regulation of telomerase reverse transcriptase expression in differentiating human teratocarcinoma cells, *Biochem. Biophys. Res. Commun.* 306 (3) (2003) 650–659.
- [92] M.I. Aladjem, et al., ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage, *Curr. Biol.* 8 (3) (1998) 145–155.
- [93] T. Burdon, A. Smith, P. Savatier, Signalling, cell cycle and pluripotency in embryonic stem cells, *Trends Cell. Biol.* 12 (9) (2002) 432–438.
- [94] S. Prost, et al., p53-independent DNA repair and cell cycle arrest in embryonic stem cells, *FEBS Lett.* 425 (3) (1998) 499–504.
- [95] S. Zimmermann, U.M. Martens, Telomere dynamics in hematopoietic stem cells, *Curr. Mol. Med.* 5 (2) (2005) 179–185.
- [96] T. Vulliamy, et al., The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita, *Nature* 413 (6854) (2001) 432–435.
- [97] T. Vulliamy, et al., Association between aplastic anaemia and mutations in telomerase RNA, *Lancet* 359 (9324) (2002) 2168–2170.
- [98] H.W. Lee, et al., Essential role of mouse telomerase in highly proliferative organs, *Nature* 392 (6676) (1998) 569–574.
- [99] K.L. Rudolph, et al., Longevity, stress response, and cancer in aging telomerase-deficient mice, *Cell* 96 (5) (1999) 701–712.
- [100] E. Herrera, et al., Disease states associated with telomerase deficiency appear earlier in mice with short telomeres, *EMBO J.* 18 (11) (1999) 2950–2960.
- [101] A. Satyanarayanan, et al., Telomere shortening impairs organ regeneration by inhibiting cell cycle re-entry of a subpopulation of cells, *EMBO J.* 22 (15) (2003) 4003–4013.
- [102] S.E. Artandi, et al., Constitutive telomerase expression promotes mammary carcinomas in aging mice, *Proc. Natl. Acad. Sci. U. S. A.* 99 (12) (2002) 8191–8196.
- [103] A. Canela, et al., Constitutive expression of tert in thymocytes leads to increased incidence and dissemination of T-cell lymphoma in Lck-Tert mice, *Mol. Cell. Biol.* 24 (10) (2004) 4275–4293.
- [104] E. Gonzalez-Suarez, et al., Antagonistic effects of telomerase on cancer and aging in K5-mTert transgenic mice, *Oncogene* 24 (13) (2005) 2256–2270.
- [105] P.C. Nowell, The clonal evolution of tumor cell populations, *Science* 194 (4260) (1976) 23–28.
- [106] D.J. Argyle, T. Blacking, From viruses to cancer stem cells: dissecting the pathways to malignancy, *Vet. J.* 177 (3) (2008) 311–323.
- [107] D. Bonnet, J.E. Dick, Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell, *Nat. Med.* 3 (7) (1997) 730–737.
- [108] M. Al-Hajj, et al., Prospective identification of tumorigenic breast cancer cells, *Proc. Natl. Acad. Sci. U. S. A.* 100 (7) (2003) 3983–3988.
- [109] S. Bao, et al., Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor, *Cancer Res.* 66 (16) (2006) 7843–7848.
- [110] S.K. Singh, et al., Identification of human brain tumour initiating cells, *Nature* 432 (7015) (2004) 396–401.
- [111] M.D. Taylor, et al., Radial glia cells are candidate stem cells of ependymoma, *Cancer Cell* 8 (4) (2005) 323–335.

- [112] N. Uchida, et al., Direct isolation of human central nervous system stem cells, *Proc. Natl. Acad. Sci. U. S. A.* 97 (26) (2000) 14720–14725.
- [113] C.A. O'Brien, et al., A human colon cancer cell capable of initiating tumour growth in immunodeficient mice, *Nature* 445 (7123) (2007) 106–110.
- [114] L. Ricci-Vitiani, et al., Identification and expansion of human colon-cancer-initiating cells, *Nature* 445 (7123) (2007) 111–115.
- [115] P.C. Hermann, et al., Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer, *Cell Stem. Cell.* 1 (3) (2007) 313–323.
- [116] G. Iannolo, et al., Apoptosis in normal and cancer stem cells, *Crit. Rev. Oncol. Hematol.* 66 (1) (2008) 42–51.
- [117] Y. Atlasi, et al., OCT-4, an embryonic stem cell marker, is highly expressed in bladder cancer, *Int. J. Cancer* 120 (7) (2007) 1598–1602.
- [118] M.E. Prince, et al., Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma, *Proc. Natl. Acad. Sci. U. S. A.* 104 (3) (2007) 973–978.
- [119] C.P. Gibbs, et al., Stem-like cells in bone sarcomas: implications for tumorigenesis, *Neoplasia* 7 (11) (2005) 967–976.
- [120] D. Fang, et al., A tumorigenic subpopulation with stem cell properties in melanomas, *Cancer Res.* 65 (20) (2005) 9328–9337.
- [121] G.M. Seigel, et al., Cancer stem cell characteristics in retinoblastoma, *Mol. Vis.* 11 (2005) 729–737.
- [122] S. Rizzo, G. Attard, D.L. Hudson, Prostate epithelial stem cells, *Cell Prolif.* 38 (6) (2005) 363–374.
- [123] K. Ishikawa, et al., A case of an alpha-fetoprotein-producing intrahepatic cholangiocarcinoma suggests probable cancer stem cell origin, *Oncologist* 12 (3) (2007) 320–324.
- [124] M. Gutova, et al., Identification of uPAR-positive chemoresistant cells in small cell lung cancer, *PLoS ONE* 2 (2) (2007) e243.
- [125] A. Cozzio, et al., Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors, *Genes Dev.* 17 (24) (2003) 3029–3035.
- [126] K. Takahashi, et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, *Cell* 131 (5) (2007) 861–872.
- [127] I. Ben-Porath, et al., An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors, *Nat. Genet.* 40 (5) (2008) 499–507.
- [128] D.L. Jones, A.J. Wagers, No place like home: anatomy and function of the stem cell niche, *Nat. Rev. Mol. Cell. Biol.* 9 (1) (2008) 11–21.
- [129] C. Calabrese, et al., A perivascular niche for brain tumor stem cells, *Cancer Cell* 11 (1) (2007) 69–82.
- [130] J.B. Sneddon, et al., Bone morphogenetic protein antagonist gremlin 1 is widely expressed by cancer-associated stromal cells and can promote tumor cell proliferation, *Proc. Natl. Acad. Sci. U. S. A.* 103 (40) (2006) 14842–14847.
- [131] H. Wilson, et al., Isolation and characterisation of cancer stem cells from canine osteosarcoma, *Vet. J.* 175 (1) (2008) 69–75.
- [132] C. Penzo, T. Blacking, R. Muirhead, R. Else, D.J. Argyle, Isolation and characterization of putative feline mammary cancer initiating cells. *Veterinary and Comparative Oncology* (In press).
- [133] M. Athar, et al., Hedgehog signalling in skin development and cancer, *Exp. Dermatol.* 15 (9) (2006) 667–677.
- [134] G. Bhardwaj, et al., Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation, *Nat. Immunol.* 2 (2) (2001) 172–180.
- [135] G.P. Dotto, Notch tumor suppressor function, *Oncogene* 27 (38) (2008) 5115–5123.
- [136] R. Fodde, T. Brabletz, Wnt/beta-catenin signaling in cancer stemness and malignant behavior, *Curr. Opin. Cell. Biol.* 19 (2) (2007) 150–158.
- [137] M.A. Goodell, et al., Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo, *J. Exp. Med.* 183 (4) (1996) 1797–1806.
- [138] M. Kim, et al., The multidrug resistance transporter ABCG2 (breast cancer resistance protein 1) effluxes Hoechst 33342 and is overexpressed in hematopoietic stem cells, *Clin. Cancer Res.* 8 (1) (2002) 22–28.
- [139] J. Lessard, G. Sauvageau, Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells, *Nature* 423 (6937) (2003) 255–260.
- [140] C. Leung, et al., Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas, *Nature* 428 (6980) (2004) 337–341.
- [141] N.A. Lobo, et al., The biology of cancer stem cells, *Annu. Rev. Cell. Dev. Biol.* 23 (2007) 675–699.
- [142] I. Malanchi, et al., Cutaneous cancer stem cell maintenance is dependent on beta-catenin signalling, *Nature* 452 (7187) (2008) 650–653.
- [143] A.V. Molofsky, et al., Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation, *Nature* 425 (6961) (2003) 962–967.
- [144] V. Palma, et al., Sonic hedgehog controls stem cell behavior in the postnatal and adult brain, *Development* 132 (2) (2005) 335–344.
- [145] I.K. Park, S.J. Morrison, M.F. Clarke, Bmi1, stem cells, and senescence regulation, *J. Clin. Invest.* 113 (2) (2004) 175–179.
- [146] I.K. Park, et al., Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells, *Nature* 423 (6937) (2003) 302–305.
- [147] T. Reya, H. Clevers, Wnt signalling in stem cells and cancer, *Nature* 434 (7035) (2005) 843–850.
- [148] A. Ruiz i Altaba, P. Sanchez, N. Dahmane, Gli and hedgehog in cancer: tumours, embryos and stem cells, *Nat. Rev. Cancer* 2 (5) (2002) 361–372.
- [149] S.M. Amarnath, et al., In vitro quantification of the cytotoxic T lymphocyte response against human telomerase reverse transcriptase in breast cancer, *Int. J. Oncol.* 25 (1) (2004) 211–217.
- [150] S. Bao, et al., Glioma stem cells promote radioresistance by preferential activation of the DNA damage response, *Nature* 444 (7120) (2006) 756–760.
- [151] D.M. Berman, et al., Medulloblastoma growth inhibition by hedgehog pathway blockade, *Science* 297 (5586) (2002) 1559–1561.
- [152] A.E. Bilsland, et al., Selective ablation of human cancer cells by telomerase-specific adenoviral suicide gene therapy vectors expressing bacterial nitroreductase, *Oncogene* 22 (3) (2003) 370–380.
- [153] T. Boon, P. van der Bruggen, Human tumor antigens recognized by T lymphocytes, *J. Exp. Med.* 183 (3) (1996) 725–729.
- [154] A.M. Burger, et al., The G-quadruplex-interactive molecule BRACO-19 inhibits tumor growth, consistent with telomere targeting and interference with telomerase function, *Cancer Res.* 65 (4) (2005) 1489–1496.
- [155] K. Damm, et al., A highly selective telomerase inhibitor limiting human cancer cell proliferation, *Embo J.* 20 (24) (2001) 6958–6968.
- [156] Z.G. Dikmen, et al., In vivo inhibition of lung cancer by GRN163L: a novel human telomerase inhibitor, *Cancer Res.* 65 (17) (2005) 7866–7873.
- [157] X. Fan, et al., Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors, *Cancer Res.* 66 (15) (2006) 7445–7452.
- [158] G. Feldmann, et al., Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers, *Cancer Res.* 67 (5) (2007) 2187–2196.
- [159] D.E. Gomez, A.M. Tejera, O.A. Olivero, Irreversible telomere shortening by 3'-azido-2',3'-dideoxythymidine (AZT) treatment, *Biochem. Biophys. Res. Commun.* 246 (1) (1998) 107–110.
- [160] M.M. Gottesman, T. Fojo, S.E. Bates, Multidrug resistance in cancer: role of ATP-dependent transporters, *Nat. Rev. Cancer* 2 (1) (2002) 48–58.
- [161] A.E. Hochreiter, et al., Telomerase template antagonist GRN163L disrupts telomere maintenance, tumor growth, and metastasis of breast cancer, *Clin. Cancer Res.* 12 (10) (2006) 3184–3192.
- [162] W.N. Keith, et al., Drug insight: cancer cell immortality-telomerase as a target for novel cancer gene therapies, *Nat. Clin. Pract. Oncol.* 1 (2) (2004) 88–96.
- [163] M.R. Parkhurst, et al., Immunization of patients with the hTERT:540–548 peptide induces peptide-reactive T lymphocytes that do not recognize tumors endogenously expressing telomerase, *Clin. Cancer Res.* 10 (14) (2004) 4688–4698.
- [164] S.G. Piccirillo, et al., Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells, *Nature* 444 (7120) (2006) 761–765.
- [165] J.A. Plumb, et al., Telomerase-specific suicide gene therapy vectors expressing bacterial nitroreductase sensitize human cancer cells to the pro-drug CB1954, *Oncogene* 20 (53) (2001) 7797–7803.
- [166] E. Salvati, et al., Telomere damage induced by the G-quadruplex ligand RHPS4 has an antitumor effect, *J. Clin. Invest.* 117 (11) (2007) 3236–3247.
- [167] A.H. Schinkel, et al., Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs, *Cell* 77 (4) (1994) 491–502.
- [168] A. Shaw, W. Bushman, Hedgehog signaling in the prostate, *J. Urol.* 177 (3) (2007) 832–838.
- [169] J.W. Shay, W.E. Wright, Telomerase therapeutics for cancer: challenges and new directions, *Nat. Rev. Drug. Discov.* 5 (7) (2006) 577–584.
- [170] L.V. Titu, et al., Cytotoxic T-cell immunity against telomerase reverse transcriptase in colorectal cancer patients, *Oncol. Rep.* 12 (4) (2004) 871–876.
- [171] J.E. Visvader, G.J. Lindeman, Cancer stem cells in solid tumours: accumulating evidence and unresolved questions, *Nat. Rev. Cancer* 8 (10) (2008) 755–768.
- [172] R.H. Vonderheide, et al., Vaccination of cancer patients against telomerase induces functional antitumor CD8+ T lymphocytes, *Clin. Cancer Res.* 10 (3) (2004) 828–839.
- [173] C.R. Walkley, et al., A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency, *Cell* 129 (6) (2007) 1097–1110.
- [174] S. Wang, D. Yang, M.E. Lippman, Targeting Bcl-2 and Bcl-XL with nonpeptidic small-molecule antagonists, *Semin. Oncol.* 30 (5 Suppl 16) (2003) 133–142.
- [175] S. Zhou, et al., Hematopoietic cells from mice that are deficient in both Bcrp1/Abcg2 and Mdr1a/1b develop normally but are sensitized to mitoxantrone, *Biotechniques* 35 (6) (2003) 1248–1252.
- [176] M. Paoloni, C. Khanna, Translation of new cancer treatments from pet dogs to humans, *Nat. Rev. Cancer* 8 (2) (2008) 147–156.
- [177] F. Lingaas, et al., A mutation in the canine BHD gene is associated with hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis in the German Shepherd dog, *Hum. Mol. Genet.* 12 (23) (2003) 3043–3053.
- [178] D.M. Vail, E.G. MacEwen, Spontaneously occurring tumors of companion animals as models for human cancer, *Cancer Invest.* 18 (8) (2000) 781–792.
- [179] S.J. Withrow, et al., Comparative aspects of osteosarcoma. Dog versus man, *Clin. Orthop. Relat. Res.* (270) (1991) 159–168.